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We sought to optimize the conditions under which B7-mediated gene transfer could effect anti-tumor immunity. Transduction of murine mammary carcinoma cell lines and human breast cancer cell lines with adenoviruses expressing B7-1 led to high levels of B7 expression. The expression of mB7-1 on melanoma cells but not mammary carcinoma cells results in reduced growth rate. Interestingly, adenovirus-mediated expression of mB7-1 on mammary carcinoma cells did not result in a reduced tumor growth rate. Furthermore, adenovirus-mediated expression of hB7 on breast cancer cells (e.g. MCF-7 or MCF-10 cells) did not lead to co-stimulation of allogeneic T cells *in vitro*. However, expression of B7-1 on human melanoma cells could confer to co-stimulatory functions. This suggests that tumor derived factors may play a direct role in limiting the immune response to breast cancer cells. We have shown that some human breast cancer cell lines produce soluble factors which inhibit the proliferative response of human T cells. We showed that TGFβ produced by MCF-7 cells does not account for the observed immune suppressive activity. Production of PGE_2 appears to play a significant role. The treatment of MCF-7 cells with indomethacin partially alleviates the inhibitory effect.
These results suggest that immunotherapy using intratumoral injection of Ad.hB7-1 may require other pharmacological measures for being effective for the treatment of breast cancer patients.

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FOREWORD

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Heike Boxhorn 9/12/97

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Introduction

T cell activation requires both an antigen-specific and an antigen-nonspecific signal as described by the two signal model of lymphocyte activation [1-4]. The antigen-specific signal is provided by the interaction of the T cell receptor (TCR) with peptides presented in the Major Histocompatibility Complex (MHC). A secondary signal is generated by a co-stimulatory molecule interacting with its cognate ligand on T cells, CD28. The absence of a co-stimulus results in anergy and programmed cell death of T cells [3, 5-8]. B7-1 is restricted in its expression to cells that traditionally act as antigen presenting cells, such as dendritic cells, macrophages, activated B cells and peritoneal exudate cells [9]. It is also functionally expressed on activated T cells, which suggests a possible mechanism of T cell autocrine co-stimulation [10]. It has been proposed that human tumor cells engineered to express B7-1 can be used to elicit anti-tumor immunity [11-13]. Many investigators have shown that the expression of B7-1 on murine tumors results in tumor rejection (Coughlin 95, Chen 92, Townsend 93, Allison 92, Hodge 94 , Baskar 90, Yang 95) and the induction of protective immunity [13-19]. However, the effectiveness of B7-1 expression appears to be limited by the immunogenicity of the tumor studied [11]. It has been proposed that tumor cells may escape immune mediated destruction by producing immunosuppressive factors, such as TGFβ [20], Il-10 [21], prostaglandins [22], leukotrienes [23] and other factors [24-26]. Breast carcinomas produce prostaglandins, mainly PGE_2 [Watson, 1992 #35; Fulton, 1991 #21] and PGE_2 levels are elevated in malignant breast cancer tissues [27]. PGE_2 is a metabolite of the arachidonic acid pathway that is catalyzed by cyclooxygenase. It is known as a potent immunomodulator. The immunosuppressive effects of PGE_2 include the inhibition of T cell proliferation [28, 29], the inhibition of the differentiation of lymphokine activated killer cells (LAK) , the suppression of natural killer cell (NK) activity [22, 30, 31], the downregulation of a humoral response [32] and the suppression of lymphokine production [33, 34].
In this study we show that PGE_2 but not TGFβ, both produced by MCF-7 cells, can limit the efficiency of the B7-1 mediated induction of an anti-breast cancer immune response.

Materials and Method

Adenoviral transduction

Tumor cells ($3x10^6$ cells/100 mm dish) were transduced with and Ad.hB7-1 or Ad.lacZ at a MOI of 100 in a total volume of 3.5 ml growth medium containing 2% FBS. The next day fresh growth medium was added and on day 3 transgene expression was confirmed by flow cytometry.

Flow cytometry

Analysis of the Ad.hB7-1 transduced cells by flow cytometry was performed using the BB-1/B7-1 mAb (Becton-Dickinson, CA) or a mIgG1 Isotype control followed by an goat anti-mouse IgG, FITC labeled (Sigma). Tumor cells transduced with Ad.mB7-1 were incubated with an anti-mCD80 (B7-1) antibody (Pharmingen) or a rat IgG2a Isoytype (Pharmingen) for 1 hour on ice followed by an FITC-labeled goat anti-rat IgG2a antibody. Flow cytometry of the Ad.lacZ transduced cells was carried out after incubation with FDG (fluorescein digalactosidase) (Molecular Probes) for 1minute at 37°C followed by incubation on ice for 30 minutes.

Tumorigenicity study

Three days after *in vitro* transduction of the murine breast carcinoma cell line T2994 with Ad.mB7-1 or Ad.lacZ, Balb/c mice (Charles Rivers) were injected subcutaneously in the left flank with $1x10^6$ untransduced or transduced T2994 cells (8 mice pre group). The animals were observed for tumor growth every other day and tumor sizes were monitored. Tumor volume was calculated as ($V=L \times W^2/2$). As soon as a tumor reached a size of 1 cm in largest diameter the mice were euthanized. For the tumorigenesis study and re-challenge studies with the murine melanoma K1735 cells adenoviral transduction was performed at a MOI of 1000. Cells were harvested 3 days after transduction and injected subcutaneously into the right flank of C3H mice ($1.0x10^6$ cells/mouse, 10 mice/group). Mice injected with untransduced K1735 cells ($1.0x10^6$ cells/mouse, 10 mice) served as positive controls for tumor growth. Animals surviving to day 55 were re-challenged with $1.0x10^6$ K1735 cells subcutaneously on the left flank. Naive mice injected with untransduced K1735 cells ($1.0x10^6$ cells/mouse, 5 mice) served as positive controls in the re-challenge experiments. The experiment was terminated on day 105.

Collecting conditioned medium (CM) from MCF-7 cells

MCF-7 cells ($3x10^6$ cells/100 mm dish) were cultured in 5 ml RPMI medium containing 10% heat inactivated FBS at 37°C in a for 24 hours. CM was collected, centrifuged to remove dead cells and stored in aliquots at -20°C. CM from MCF-7 cells used in the anti-TGFβ thymidine assay was collected after 3 hours incubation of $3x10^6$ MCF-7 cells in 5 ml serumfree RPMI medium.

Standard thymidine assay

Human mononuclear cells were obtained from peripheral blood by Ficoll density centrifugation (Pharmacia). The cells were plated at a concentration of $1x10^5$ cells per well of a 96-well round bottom culture. Where indicated PHA (Sigma) was added at a concentration of 5 μg/ml or phorbol-myristate actetate (PMA) (10 ng/ml) in combination with ionomycin (360 ng/ml) were added. PGE_2 (Fluka) and the pan-specific anti-TGFβ neutralizing antibody (R&D Systems) were used in concentration indicated. Cells were cultured for 5 days, pulsed with 1 μCi thymidine and harvested 18 hours later in a scintillation counter. When tumor cells were used as stimulators, $1x10^6$ tumor cells were treated with 100 μg mitomycin C (Sigma) for 45 minutes 37°C in fresh RPMI-medium. Human T cells were purified from peripheral blood by negative selection using an monoclonal antibody mixture of mouse anti human CD14 (macrophages), CD16 (NK

cells), CD19 (on B cells) and MHC class II (L243 mAb) (gift from L. Turka, University of Pennsylvania) followed by incubation with BioMag goat anti mouse IgG magnetic beats (Perseptive Diagnostics). Tumor cells ($2.5x10^4$ cells/well) and human T cells ($1x10^5$ cells/well) were co-cultured for 5 days, pulsed with thymidine and harvested as described above.

Results

Transduction of the murine mammary carcinoma cell line T2994 and human breast cancer cell lines with Ad.mB7-1 and Ad.hB7-1 respectively, results in high level gene expression

The murine mammary carcinoma cell line T2994 does not express B7-1 in quantities that are detectable by flow cytometry (Figure 1). Upon transduction with Ad.mB7-1 or Ad.lacZ at an MOI of 100, greater than 82% of the T2994 cells expressed the transgene after three days. Similar results were obtained when the murine mammary carcinoma SCK cell line was trasnsduced (data not shown). Human breast cancer cell lines MCF-7, MCF-10, BT-20, MDA MB-231 and BT-474 transduced with Ad.hB7-1 at an MOI of 100, results in B7-1 expression on at least 91% of the cells. None of these cells normally express hB7-1 in quantities detectable by flow cytometry. Thus, breast cancer cells can be readily transduced to express B7-1 at levels sufficient to allow T cell activation.

Tumorigenicity of the murine mammary carcinoma cell line T2994 transduced with Ad.mB7-1

We sought to compare the growth behavior of untransduced T2994 cells and Ad.mB7-1 transduced T2994 cells in immunocompetent mice. T2294 cells were transduced *in vitro* as discussed above. Cells were harvested 3 days after transduction and injected subcutaneously into the right flank of Balb/c mice. Mice injected with untransduced T2994 cells served as positive controls for tumor growth. The tumor incidence curve is shown in Figure 2. All the mice developed tumors by day 25. By day 40 the experiment was terminated due to the tumor mass. There was no significant difference of the tumor growth rate in the groups receiving transduced T2994 cells and untransduced tumor cells. Adenovirus-mediated expression of B7-1 on SCK cells resulted in a retardation of the tumor growth rate with all animals developing tumors at day (data not shown). In contrast, the murine melanoma K1735 cell line transduced with Ad.mB7-1 was rejected in immunocompetent mice. None of the mice developed tumors (Figure 3). Thus, we suggested that the observed difference in the ability of B7-1 to reduce the tumor growth rate between mammary carcinoma cells and melanoma cells was independent of co-stimulation.

MCF-7 and MCF-10 human breast cancer cells transduced with Ad.hB7-1 fail to co-stimulate allogeneic T cells

To test the hypothesis that adenoviral delivered B7-1 on tumor cells can co-stimulate human allogeneic T cell proliferation *in vitro*, we transduced the human breast cancer cell lines MCF-7 and MCF-10 with Ad.hB7-1. T cells co-cultured with B7-1 expressing MCF-7 did not proliferate (Figure 4). Similarly, the expression of B7-1 on MCF-10 cells also failed to induce proliferation of allogeneic T cells (data not shown); whereas, adenovirus mediated expression of B7-1 on the human melanoma cell line WM9 stimulated allogeneic T cells to proliferate (Figure 5). Thus, adenoviral delivered B7 on melanoma cells can provide co-stimulation but the breast cancer cells appear to be a poor substrate for B7-1 mediated T cell activation.

MCF-7 cells produce soluble factors which inhibit the proliferation of allogeneic T cells stimulated by WM9Ad.hB7-1 cells

The inability of MCF-7 cells transfected with Ad.hB7-1 to co-stimulate allogeneic T cells under conditions that worked for melanoma cells, suggested the possible presence of factors that inhibit T cell proliferation. Conditioned medium (CM) from MCF-7 cells was added to allogeneic T cells that were stimulated with the human melanoma cell line WM9 expressing B7-1 (see above). WM9 cells expressing hB7-1 were able to stimulate allogeneic T cells in the absence of MCF-7 supernatant; however, the proliferation was inhibited completely in a dose response manner by the addition of CM from MCF-7 cells

(Figure 6). This indicates that MCF-7 cell produce soluble factors that inhibit T cell proliferation under these conditions.

CM from MCF-7 cells inhibits the proliferation of human MN cells stimulated with PHA

The ability of CM from MCF-7 cells to inhibit the proliferation of T cells was tested when endogenous co-stimulatory signals were provided. Human mononuclear cells (MN) cells served as a source for B7 expressing antigen presenting cells and T cells. CM from MCF-7 cells was added to human MN cells stimulated with PHA. The addition of CM from MCF-7 cells blocked the proliferation of MN cells stimulated with PHA (Figure 7). This indicates that the inhibitory effect of CM from MCF-7 is effective even when endogenous antigen presenting cells are used for co-stimulation.

The inhibitory effect of CM from MCF-7 cells takes place downstream from co-stimulation and antigen recognition

To determine whether the inhibitory activity of the CM from MCF-7 cells is independent of co-stimulation, MN cells were stimulated with PMA/ionomycin. The inhibitory capacity of CM from MCF-7 cells was examined. Activated MN cells incorporated thymidine only in the absence of CM from MCF-7 cells. The inhibitory effect of CM from MCF-7 cells was dose-dependent (Figure 8). A dilution of 1:4000 of the CM from MCF-7 cells completely blocked the proliferation of stimulated T cells; whereas, the inhibitory activity of the CM was reduced at a dilution of 1:40000. Thus, co-stimulation was not required for the inhibition of the proliferation of MN cells by CM from MCF-7 cells.

The breast cancer cell lines MCF-10 and BT-474 produce soluble factors which inhibit the proliferative response of MN cells to PHA

Other breast cancer cell lines were examined for soluble factors that inhibit the proliferation of T cells. CM from MCF-10 cells, MDA-MB-231 cells, BT-474 cells, BT-20 cells, HBL 100 cells, T47D cells and SK-Br-3 were tested for their ability to inhibit the proliferation of MN cells stimulated with PHA. CM from MDA-MB-231 cells and BT-20 cells did not significantly alter the proliferation of stimulated MN cells (Figure 9). However, CM from MCF-10 cells and BT-474 cells inhibited the proliferation of MN cells in response to PHA to the same extend as the addition of MCF-7 supernatant. Thus, the production of soluble factors by breast cancer cells is a common but not universal finding.

TGFβ does not account for the inhibitory effect of the CM from MCF-7 cells on the proliferation of MN cells in response to PHA

MCF-7 cells produce active TGFβ which is known to block T cell activation. We tested if TGFβ whether responsible for the observed inhibitory effect CM from MCF-7 cells. We found that $3x10^6$/5ml MCF-7 cells produced 578 pg/ml total TGFβ (active and latent TGFβ). MN cells were stimulated with PHA and treated with CM from MCF-7 cells containing anti-TGFβ. The amount of the TGFβ neutralizing antibody used was in excess of that needed to reduce both latent and active TGFβ produced by MCF-7 cells as determined by a luciferase assay (data not shown). The proliferation of stimulated MN cells was inhibited by 1 µl and 50 µl of CM from MCF-7 cells (Figure 10). The addition of a TGFβ pan-specific antibody did not reverse the inhibition of the proliferation of MN cells.

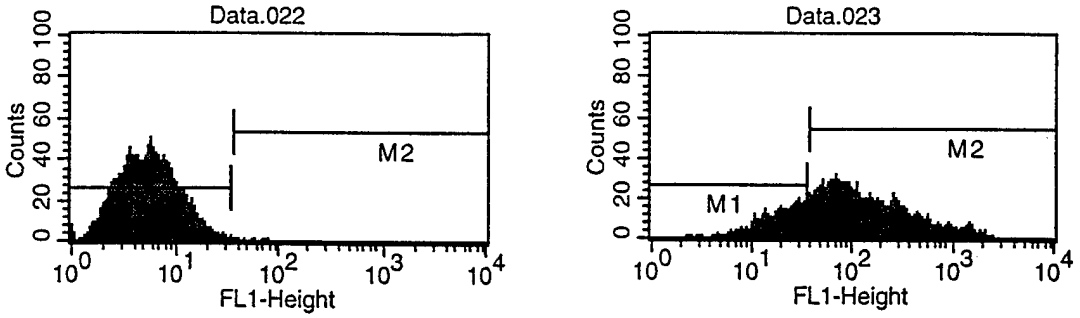
Prostaglandin E_2 (PGE_2) partially inhibits the proliferation of MN cells stimulated with PMA/ionomycin

MCF-7 cells have been shown to produce PGE_2. We investigated whether PGE_2 inhibits the proliferative response of mitogen-stimulated MN cells similarly to the observed inhibitory effect of CM from MCF-7 cells. Increasing amounts of PGE_2 (10 pg/ml to 10 µg/ml) were added to PMA/ionomycin stimulated MN cells. There was a 23% inhibition of

proliferation when PGE_2 was added at a concentration of 100 ng/ml PGE_2 and 51% inhibition when 10 μg/ml of PGE_2 was added (Figure 11). MCF-7 cells ($3x10^6$ cells) produced 200 pg/ml PGE_2 per 24 hours as determined by mass spectrometry (data not shown). Therefore, MCF-7 produce PGE_2 in quantities that are sufficient to inhibit the proliferation of MN cells.

Indomethacin treatment of MCF-7 cells partially alleviates the immune suppressive effect of the CM from MCF-7 cells

To determine if the elimination of prostaglandin E_2 (PGE_2) in the CM of MCF-7 cells reversed its immune inhibitory capacity, MCF-7 cells were treated with the cyclooxygenase inhibitor indomethacin (100 μg/ml) for 24 hours in RPMI medium containing 10% heat inactivated FBS. The PGE_2 levels produced by the supernatants of untreated and indomethacin treated MCF-7 cells were added to PMA/ionomycin stimulated MN cells at different dilutions (1/40, 1/4000, 1/40000). CM from MCF-7 cells contained 40 pg/ml PGE_2 as determined by mass spectrometry. Indomethacin reduced the PGE_2 produced by MCF-7 cells to undetectable levels (data not shown). The high proliferation of PMA/ionomycin-stimulated MN cells was inhibited dramatically when MCF-7 supernatant was added at a dilution of 1/40 independent of indomethacin treatment (Figure 12). At a dilution of 1/4000, however, the supernatant of indomethacin treated MCF-7 cells reversed the inhibitory effect. The supernatant of untreated MCF-7 cells did not inhibit the proliferation of MN cells at a dilution of 1/40000. Indomethacin in the supernatant did not influence the response of MN cells to mitogen (data not shown).



untransduced

transduced with Ad.mB7-1

Figure 1: Transduction of the murine mammary carcinoma cell line T2994 with Ad.mB7-1. Tumor cells were transduced with and Ad.hB7-1 at a MOI of 100. Three days later B7-1 expression was determined by flow cytometry using an anti-mCD80 (B7-1) antibody and a rat IgG2a Isoytype. Greater than 82 % of the T2994 cells were positive for B7-1.

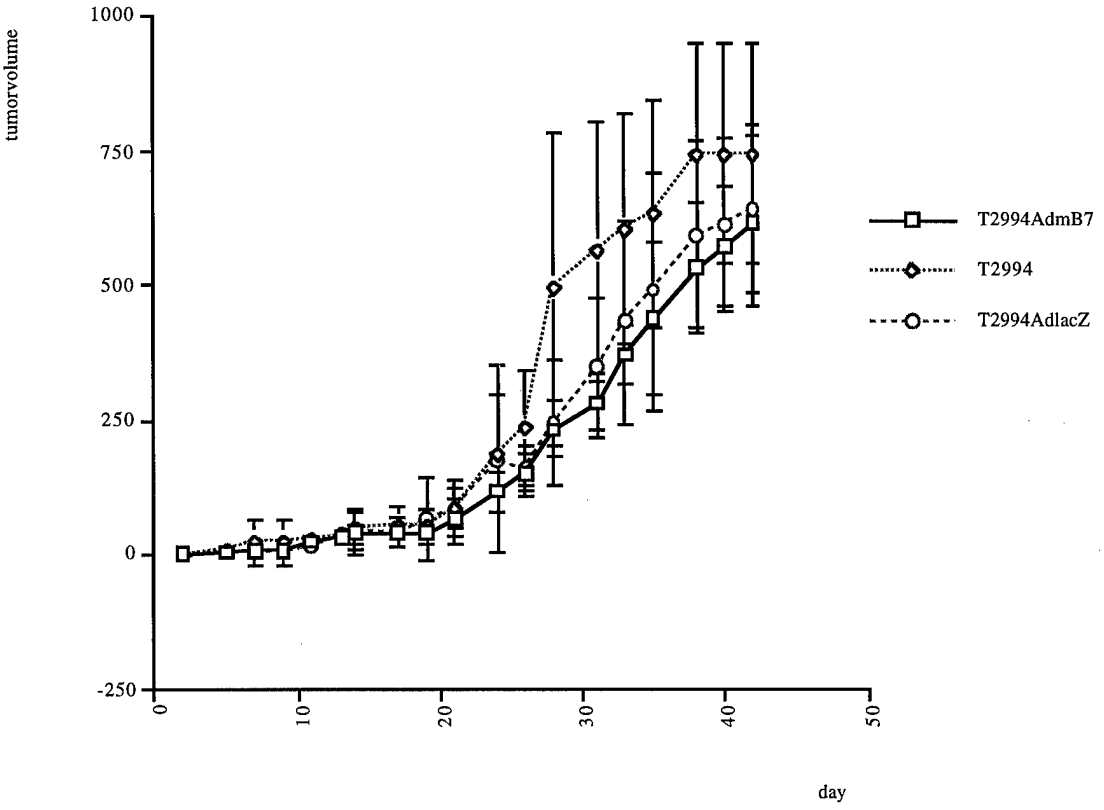


Figure 2: Tumorigenicity of the murine mammary carcinoma cell line T2994 transduced with Ad.mB7-1. Three days after in vitro transduction of the murine mammary carcinoma cell line T2994 with Ad.mB7-1 or Ad.lacZ, Balb/c mice were injected subcutaneously in the left flank with $1x10^6$ untransduced or transduced T2994 cells (8 mice pre group). The animals were observed for tumor growth every other day and tumor sizes were monitored. Tumor volume was calculated as ($V=L \times W^2/2$). As soon as a tumor reached a size of 1 cm in largest diameter the mice were euthanized. There was no significant difference of the tumor growth rate in the groups receiving untransduced and transduced T2994 cells.

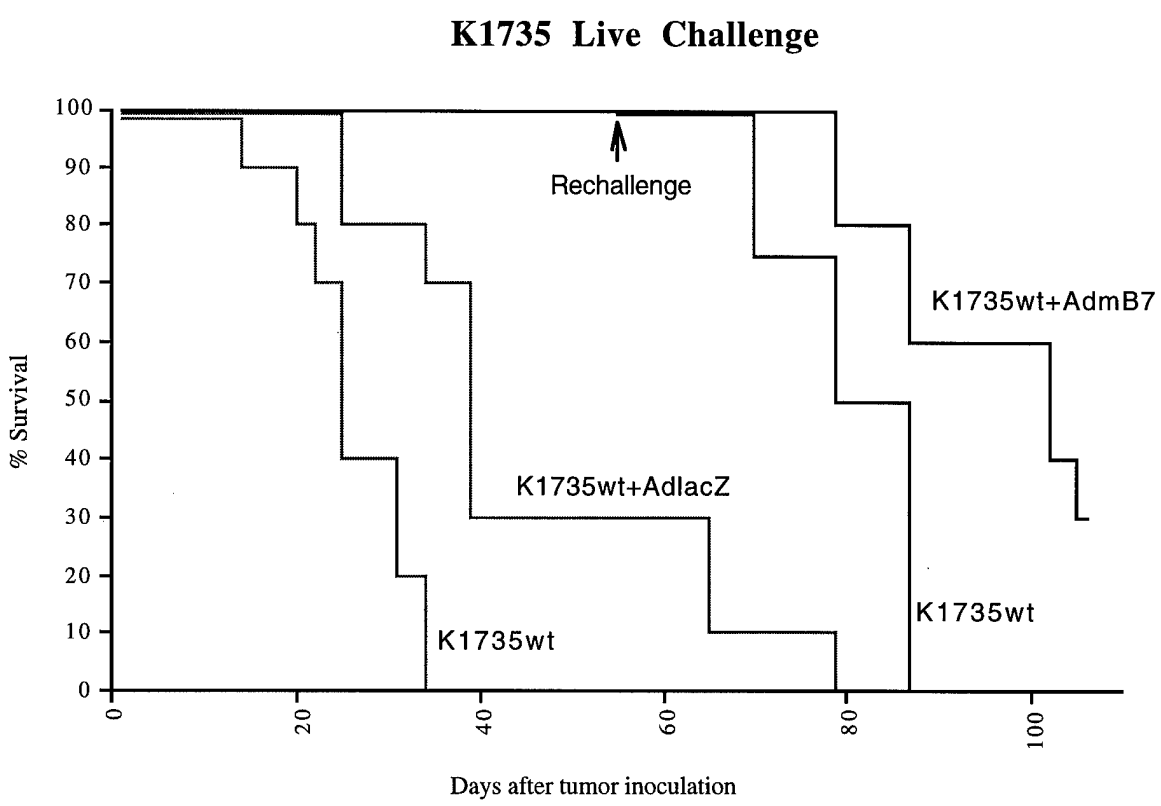


Figure 3: Survival of mice injected with the murine melanoma cell line K1735 transduced with Ad.mB7-1 and re-challenged with parental tumor cells. Untransduced and transduced cells were harvested 3 days after transduction and injected subcutaneously into the right flank of C3H mice ($1.0x10^6$ cells/mouse, 10 mice/group). Mice injected with untransduced K1735 cells ($1.0x10^6$ cells/mouse, 10 mice) served as positive controls for tumor growth. Animals surviving to day 55 were re-challenged with $1.0x10^6$ K1735 cells subcutaneously on the left flank. Naive mice injected with untransduced K1735 cells ($1.0x10^6$ cells/mouse, 5 mice) served as positive controls in the re-challenge experiments. The experiment was terminated on day 105. None of the mice receiving K1735 cells transduced with Ad.mB7-1 developed tumors.

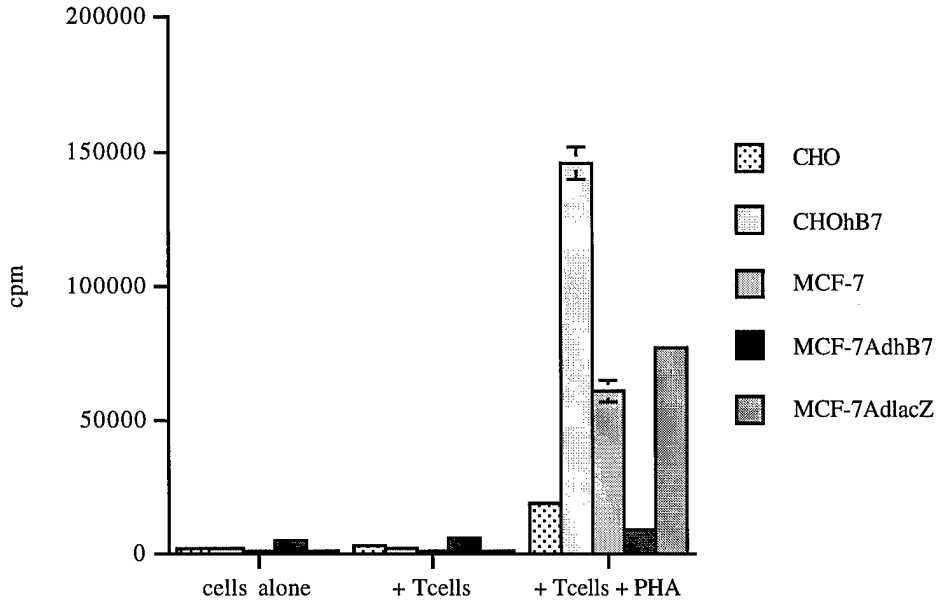


Figure 4: MCF-7 human breast cancer cells transduced with Ad.hB7-1 fail to co-stimulate allogeneic T cells. Three days after the transduction of MCF-7 cells with Ad.hB7-1 or Ad.lacZ, cells were mitomycin C treated. Untransduced and transduced cells ($2.5x10^5$ cells/well) were co-cultured with human allogeneic T cells ($1x10^5$ cells/well) for 5 days. PHA was added at a concentration of 5 μg/ml. Cells were pulsed with thymidine for 18 hours before harvesting in a scintillation counter. T cells co-cultured with B7-1 expressing MCF-7 cells did not proliferate.

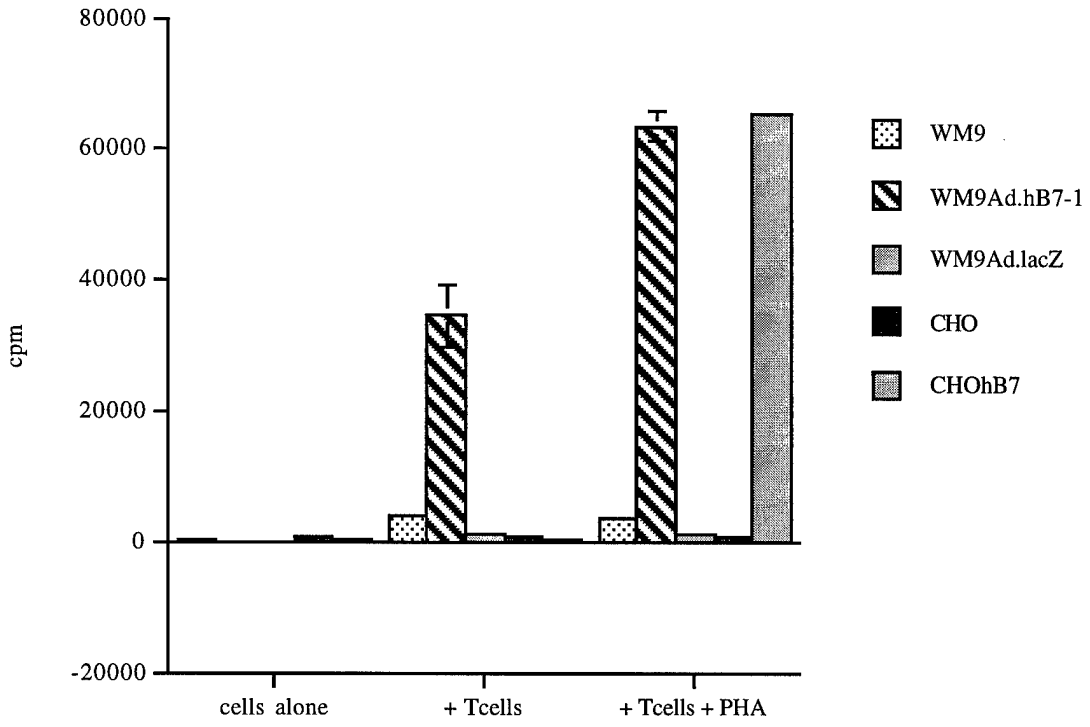


Figure 5: The human melanoma cell line WM9 transduced with Ad.hB7-1 co-stimulates allogeneic T cells. Three days after the transduction of WM9 cells with Ad.hB7-1 or Ad.lacZ, cells were mitomycin C treated. Untransduced and transduced cells ($2.5x10^5$ cells/well) were co-cultured with human allogeneic T cells ($1x10^5$ cells/well) for 5 days. PHA was added at a concentration of 5 μg/ml. Cells were pulsed with thymidine for 18 hours before harvesting in a scintillation counter. The adenovirus-mediated expression of hB7-1 on the human melanoma cell line WM9 stimulated allogeneic T cells to proliferate.

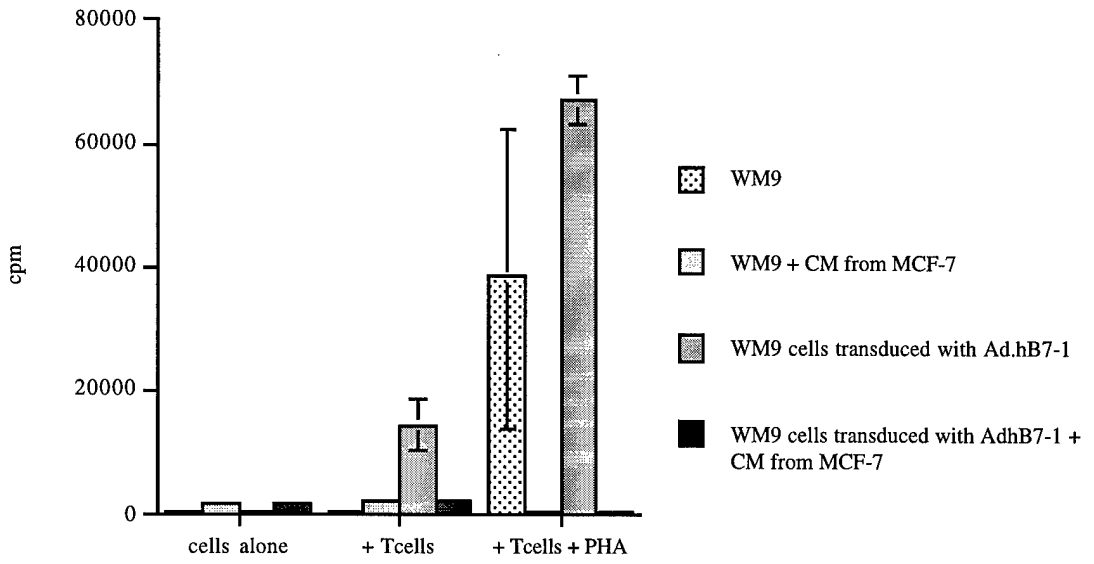


Figure 6: Addition of CM from MCF-7 cells to T cells stimulated by WM9 cells transduced with Ad.hB7-1. Three days after the transduction of WM9 cells with Ad.hB7-1 or Ad.lacZ, cells were mitomycin C treated. Untransduced and transduced cells ($2.5x10^5$ cells/well) were co-cultured with human allogeneic T cells ($1x10^5$ cells/well) for 5 days. PHA was added at a concentration of 5 μg/ml. CM from MCF-7 cells was added (50 μl) at the initiation of the co-cultures. Cells were pulsed with thymidine for 18 hours before harvesting in a scintillation counter. MCF-7 cells produce soluble factors which inhibit the proliferation of T cells stimulated by WM9Ad.hB7-1 cells.

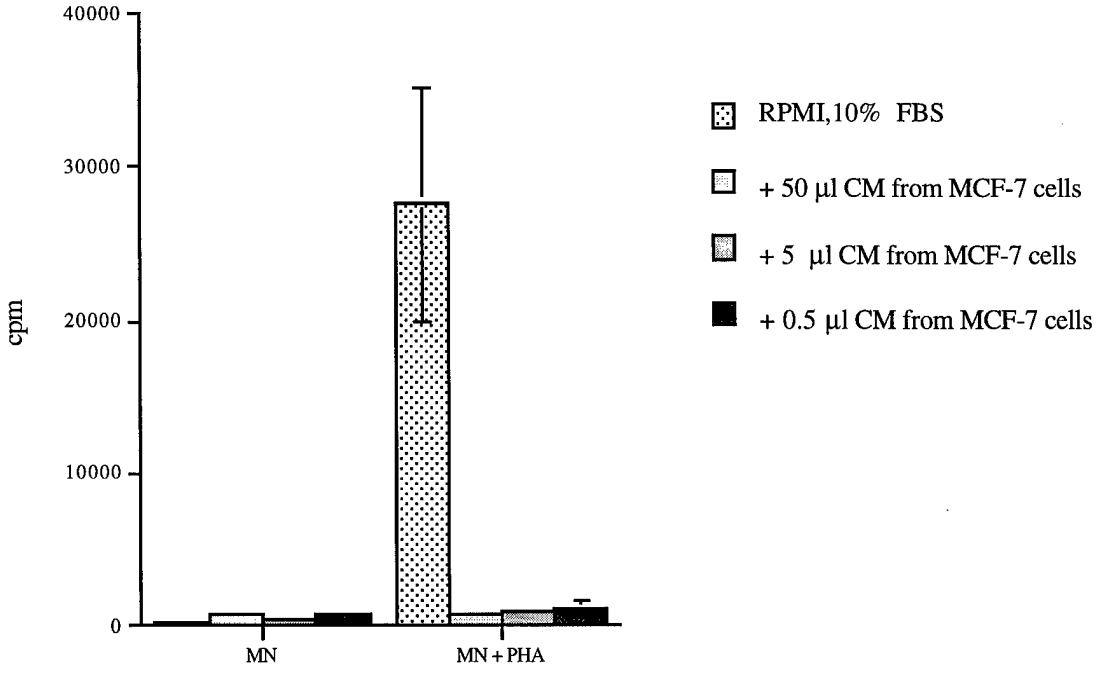


Figure 7: Addition of CM from MCF-7 cells to MN cells stimulated with PHA. Human MN cells ($1x10^5$ cells/well) were cultured in 96-well-plates for 5 days. PHA was added at a concentration of 5 µg/ml. CM from MCF-7 cells was added at the initiation of the culture. Cells were pulsed with thymidine and harvested in a scintillation counter. CM from MCF-7 cells inhibits the proliferation of MN cells stimulated by PHA.

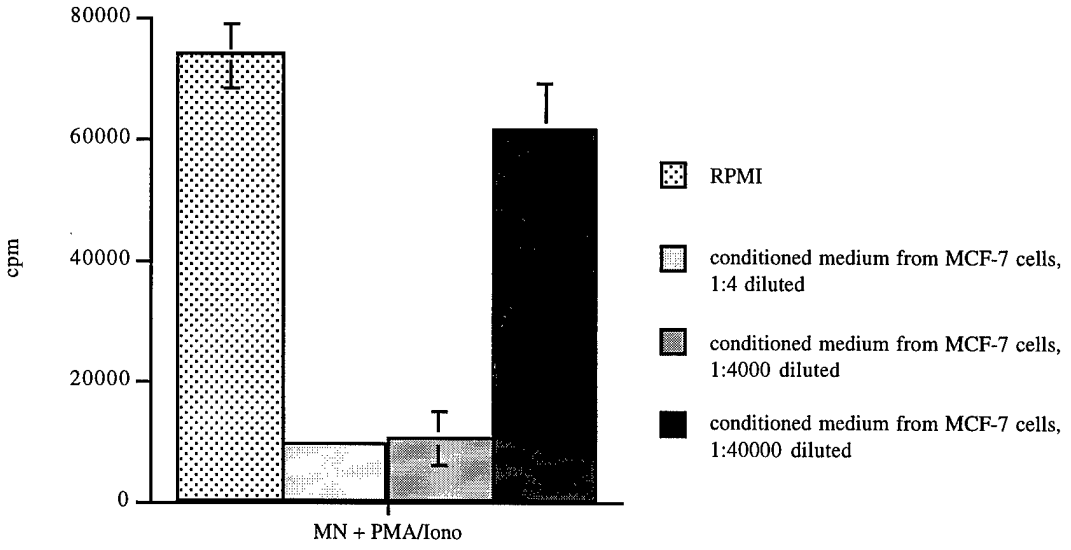


Figure 8: Addition of CM from MCF-7 cells to MN cells stimulated with PMA/ionomycin. Human MN cells ($1x10^5$ cells/well) were cultured in 96-well-plates for 5 days. PMA (10 ng/ml) and ionomycin (360 ng/ml) were added where indicated. CM from MCF-7 cells was added at the initiation of the culture. Cells were pulsed with thymidine and harvested in a scintillation counter. CM from MCF-7 cells inhibits the proliferation of MN cells stimulated by PHA. The inhibitory effect of CM from MCF-7 cells takes place downstream from co-stimulation and antigen recognition.

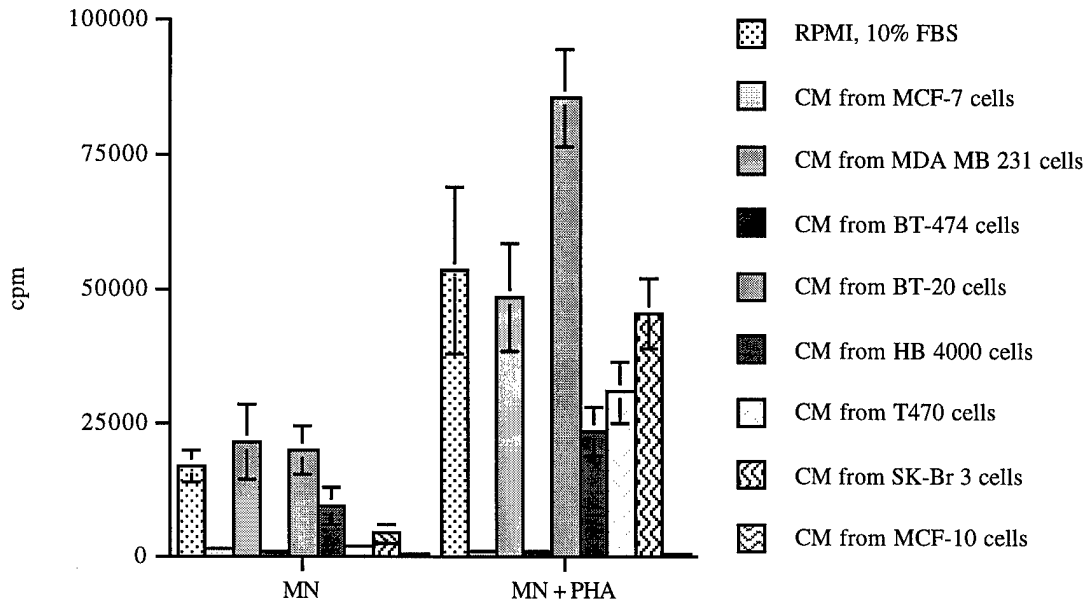


Figure 9: Addition of CM from different human breast cancer cells to MN cells stimulated with PHA. Human MN cells ($1x10^5$ cells/well) were cultured in 96-well-plates for 5 days. PHA was added at a concentration of 5 µg/ml. CM from different human breast cancer cells were added at the initiation of the culture. Cells were pulsed with thymidine and harvested in a scintillation counter. The breast cancer cell lines MCF-10 and BT-474 produce soluble factors that inhibit the proliferative response.

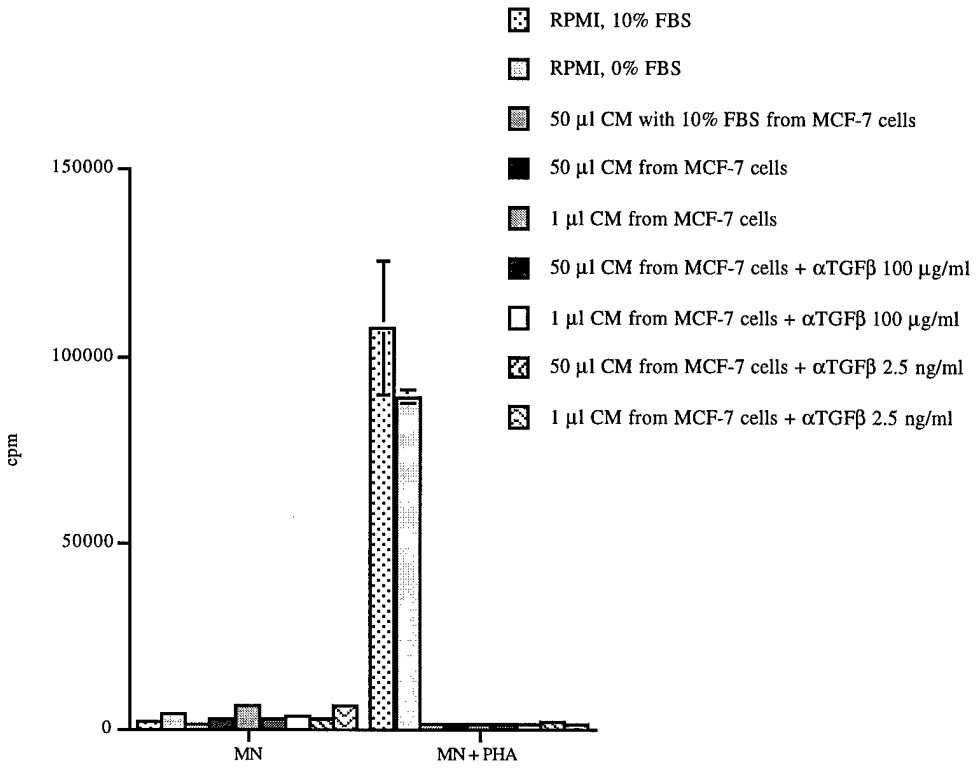


Figure 10: Testing the effect of CM from MCF-7 cells after incubation with an anti-TGFβ neutralizing antibody. Human MN cells ($1x10^5$ cells/well) were stimulated with PHA (5 µg/ml). CM from MCF-7 cells was obtained by incubating $3x10^6$ cells in 5 ml of serumfree PRMI-medium for 3 hours. CM from MCF-7 cells were added at different dilutions in the presence or absence of a pan-specific TGFβ antibody (2.5 ng/ml or 100 µg/ml). Cells were pulsed with thymidine and harvested in a scintillation counter. TGFβ does not account for the inhibitory effect of the CM from MCF-7 cells.

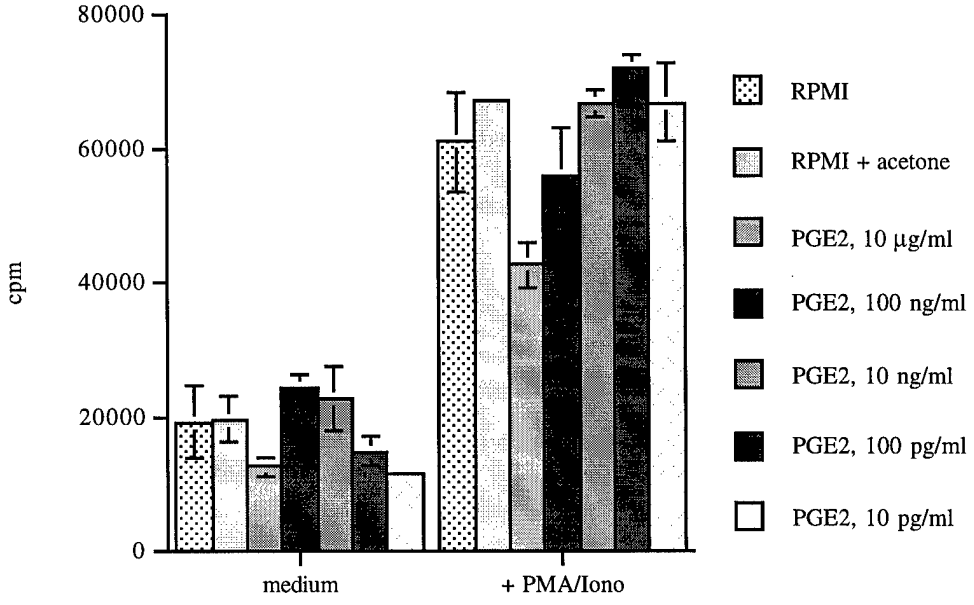


Figure 11: Addition of PGE_2 to MN cells stimulated by PMA/ionomycin. Human MN cells were stimulated with PMA (10 ng/ml) and ionomycin (360 ng/ml). PGE_2 was added at different concentrations ranging from 10 µg/ml to 10 pg/ml. PGE_2 partially inhibits the proliferation of MN cells stimulated with PMA/ionomycin.

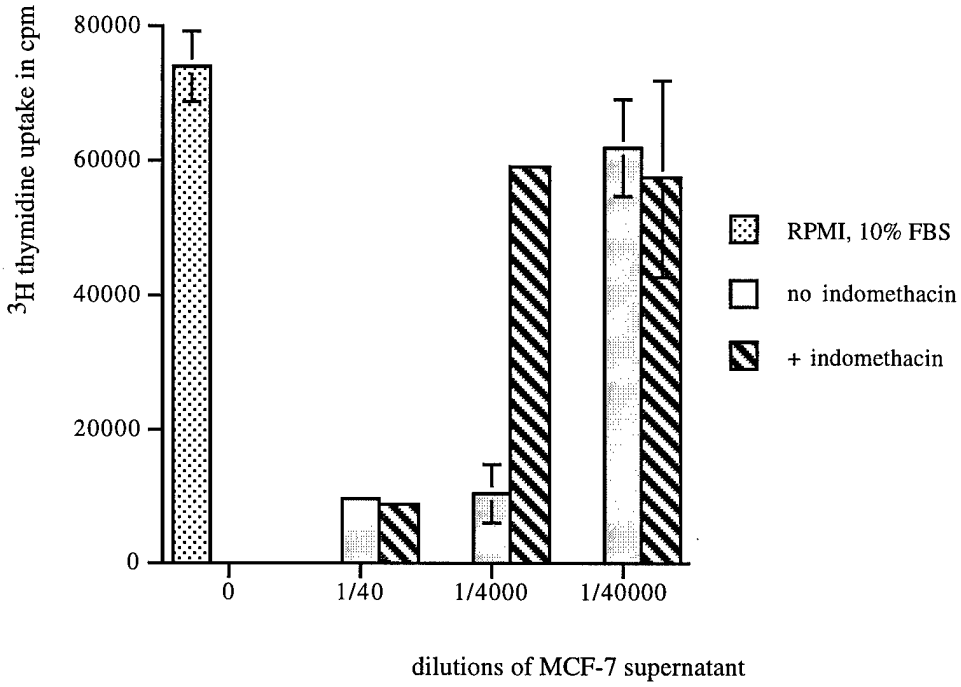


Figure 12: Testing the effect of CM MCF-7 cells treated with indomethacin. Human MN cells were stimulated with PMA (10 ng/ml) and ionomycin (360 ng/ml). MCF-7 cells were treated with indomethacin (100 μg/ml) for 24 hours. CM from untreated and indomethacin treated MCF-7 cells were added at the initiation of the culture. Cells were pulsed with thymidine and harvested in a scintillation counter. Indomethacin treatment of MCF-7 cells partially alleviates the immune suppressive effect of the CM from MCF-7 cells.

Discussion

This study was prompted by the findings from us and other investigators [13-19] that B7-1 expression can elicit an anti-tumor immune response. The efficacy of B7-mediated immunotherapy is depending on the tumor system studied. The outcome of a B7-induced anti-tumor immunity correlates with the immunogenicity of the tumor cells [11]. To confer these findings into a clinical application for breast cancer, efficient gene transfer *in vivo* is required. Adenoviral vectors are suitable vehicles to deliver B7-1 into human breast cancer cells because they transduce dividing and non-dividing cells and they yield high levels gene expression. We have shown that murine mammary carcinoma cells and human breast cancer cell lines are very susceptible to adenoviral transduction. Nevertheless, B7-1 expressing mammary carcinoma cells fail to induce an anti-tumor response *in vivo*. We hypothesized this failure may be in part due to the breast cancer cell derived immunosuppressive factor PGE_2.

PGE_2 is produced by a variety of tumors, including human breast cancer cells [35-37]. PGE_2 controls the growth of breast cancer cells in an autocrine fashion [38, 39]. However, there are conflicting data whether PGE_2 inhibits or promotes tumor cell replication [39, 40]. The effect of PGE_2 on tumor growth regulation is dependent of the stage of differentiation [41]. It has been shown that during mammary cancer progression the PGE_2 receptor is lost or altered resulting in advanced tumorigenesis. The inability of signal transduction through the PGE_2 receptor in advanced mammary tumors could explain the elevated levels of PGE_2 in tumor cells in a progressed stage of the disease [41]. Thus, the level of PGE_2 determines the stage of disease in breast cancer [27, 35]. Additionally, PGE_2 plays a role in mammary tumor metastasis [37]. The effects of PGE_2 in the immune system are mainly suppressive, although some immune stimulatory effects, such as the stimulation of Il-4 mediated class switch of B cells are described [42]. The immune suppressive functions of PGE_2 include the inhibition of T cell proliferation [28, 29], the inhibition of the differentiation of lymphokine activated killer cells (LAK) , the suppression of natural killer cell (NK) activity [22, 30, 31], the downregulation of a humoral response [32] and the suppression of lymphokine production [33, 34]. We sought to demonstrate a direct effect of tumor derived PGE_2 on T cell activation. Indomethacin, an inhibitor of prostaglandin synthesis could not completely eliminate the immunosuppressive factors produced by the human breast cancer cell line MCF-7 suggesting the presence of other inhibitory factors. A number of immune inhibitory factors have been described, such as TGFβ, IL-10, leukotrienes and others. We are currently characterizing further immunosuppressive factors. We suggest that not only the expression of specific antigens, MHC molecules, co-stimulatory and accessory molecules on tumor cells but also the secretion of immunoregulatory molecules determines the immunogenicity of breast cancer cells.

Recommendations in relation to the Statement of Work outlined in the proposal

We showed that murine mammary carcinoma cells and human breast cancer cells can be efficiently transduced with Ad.mB7-1 and Ad.hB7-1, respectively (task 1 and 2 of the Statement of Work). We compared the in vivo growth behavior of untransduced and Ad.mB7-1 transduced mammary carcinoma cells in immunocompetent mice (task 5). T cell stimulation assays with transduced human breast cancer cells were performed to determine an anti-tumor response in vitro (task 9). Interestingly, B7 expression failed to reduce the tumorigenesis in vivo and to induce co-stimulation in vitro. Therefore, we hypothesized that human breast cancer cells produce immunoinhibitory factors. Our studies indicate that the tumor derived PGE2 partially inhibits T cell proliferation. We are currently characterizing other breast cancer derived immunoinhibitory factors.

Conclusions

We conclude from our study that there is a difference in the efficacy of adenovirus-mediated expression of B7-1 on melanoma cells and breast cancer cells. The inability of B7-1 expressing mammary carcinoma cells in reducing the tumor growth rate *in vivo* and the inability of B7-1 expressing breast cancer cell lines to induce T cell proliferation *in vitro* may in part be a consequence of the production of PGE_2 and other immunosuppressive factors. We are currently characterizing the breast cancer derived factors that are responsible for the observed inhibition of the proliferation of MN cells.

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